Figure 1: Examples of Nuclease Stable Ribozyme Motifs

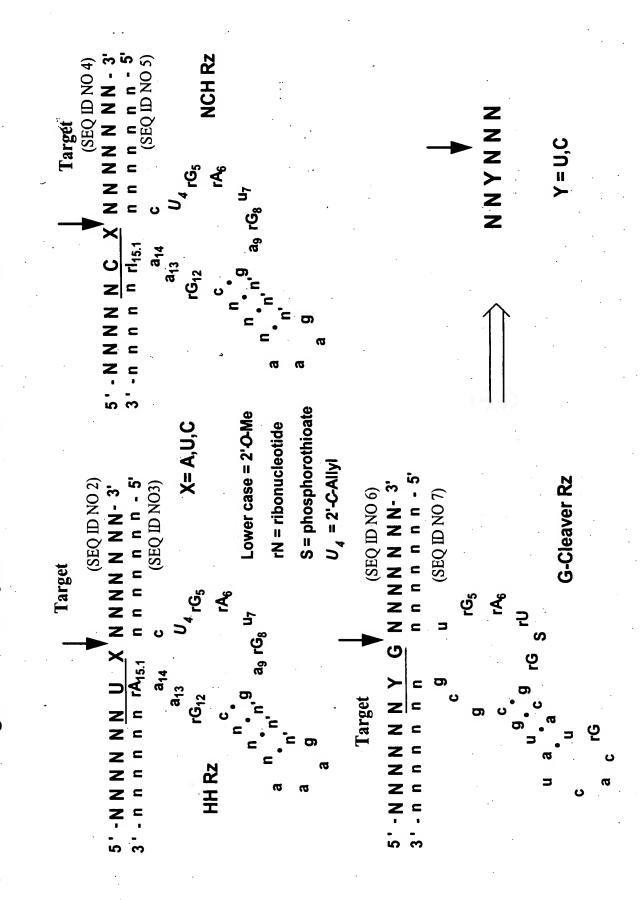


Figure 2: 2'-O-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

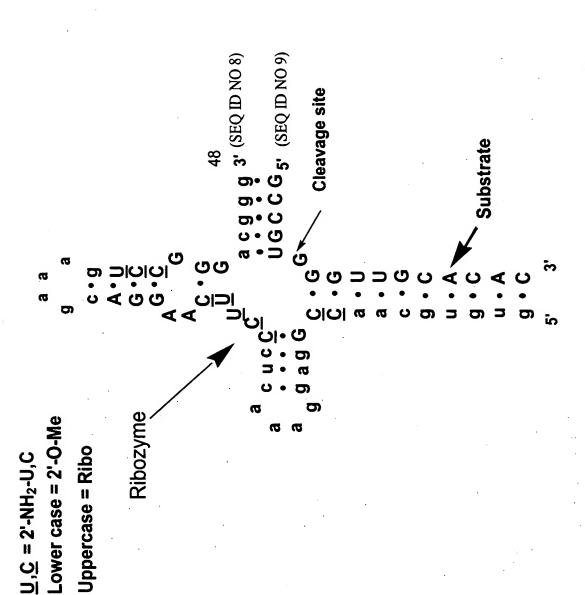
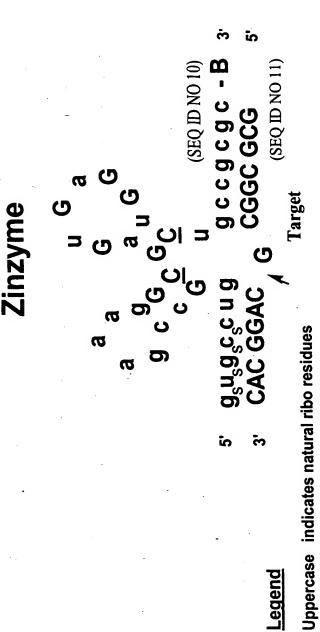


Figure 3: Stabilized Zinzyme Ribozyme Motif



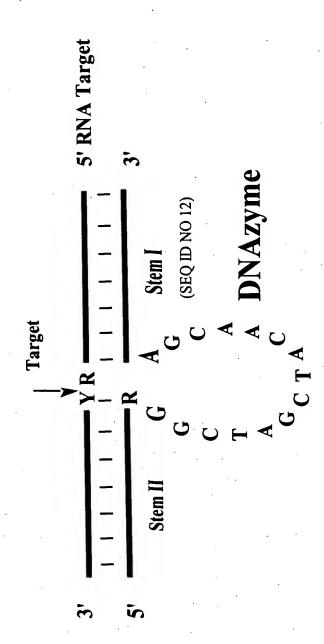
C indicates 2' - dexoy-2'-amino Cytidine

Lowercase indicates 2'-0-methyl

S indicates phosphorothioate/phosphorodithioate

B: 3'-3' abasic moiety

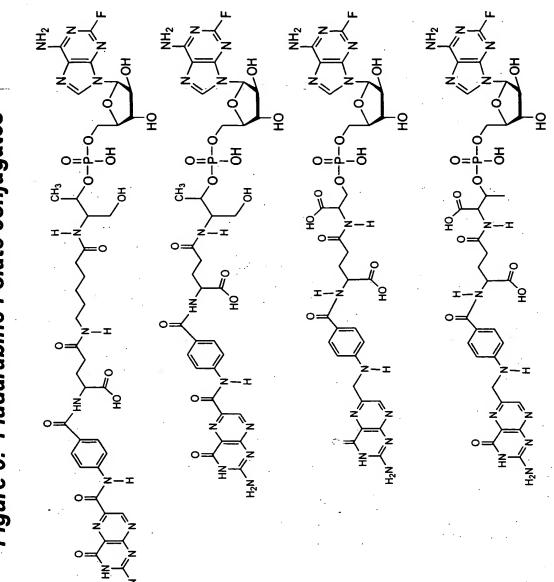
Figure 4: DNAzyme Motif



Y = U or C R = A or G

Figure 5: Synthesis of Folate Linked phosphoramidite

Figure 6: Fludarabine-Folate conjugates



8

S

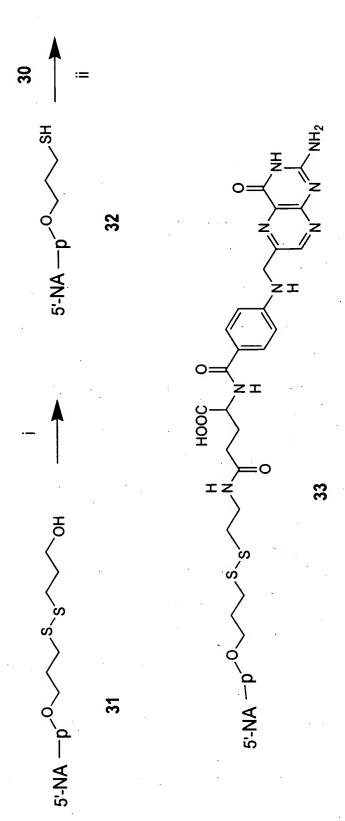
Ω

Figure 7: Solid Phase Post-synthetic conjugation of pteroic acid

Figure 8: Chemo-enzymatic synthesis of pteroic acid synthon

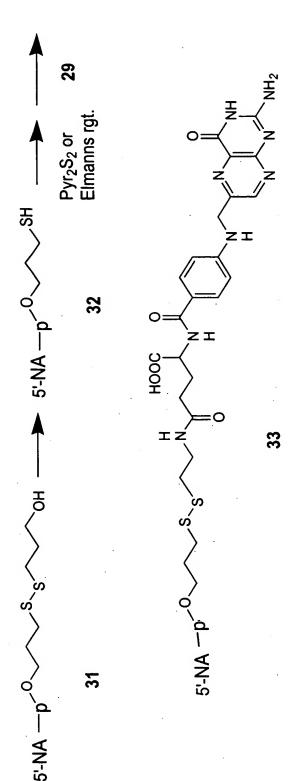
Figure 9

Figure 10



NA = Nucleic Acid, such as siNA, antisense, or enzymatic nucleic acid p = phosphorous moiety

Figure 11



NA = Nucleic Acid, such as siNA, antisense, or enzymatic nucleic acid p = phosphorous moiety

N/Pr₂POCH₂CH₂CN

Figure 13: Synthesis of N-acetyl-D-galactosamine-2'-aminouridine "DMTO conjugate

N,N-diisopropylchlorophosphoramidite, 1-methylimidazole, DIPEA, CH₂Cl₂, (iv) Ac₂O, TEA, CH₃CN, (v) Reagents and Conditions: (i) diethylamine, DMF, (ii) 8, diisopropylethylamine, DMF, (iii) 2-cyanoethyl HCI, Ac_2O , (vi) $Hg(CN)_2$, MS 4A, CH_3NO_2 -toluene 1:1, (vii) H_2 , 5% Pd-C, ethanol, (viii)

N-hydroxysuccinimide, DCC, THF.

Figure 14: Synthesis of N-acetyl-D-galactosamine-D-threoninol conjugate

HO NH Thac3NAcGa H₃C
$$\frac{1}{11}$$
 $\frac{1}{11}$ $\frac{1}{11}$

Reagents and Conditions: (i) 7, DCC, N-hydroxysuccinimide, (ii) MMTr-Cl, pyridine, (iii) 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, 1-methylimidazole, DIPEA, CH₂Cl₂.

Figure 15: Conjugation of targeting ligands to the 5'-end of a Ribozyme or siNA molecule

N-acetyl-D-galactosamine conjugate

Figure 16: Synthesis of dodecanoic acid linker

Figure 17: Oxime linked Nucleic Acid/Peptide Conjugate

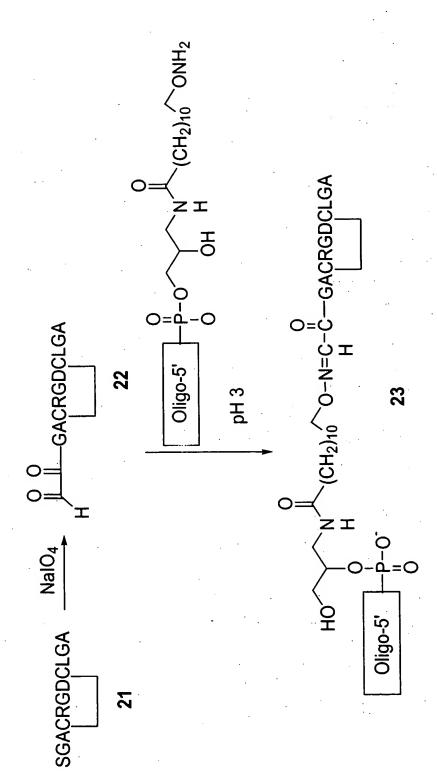
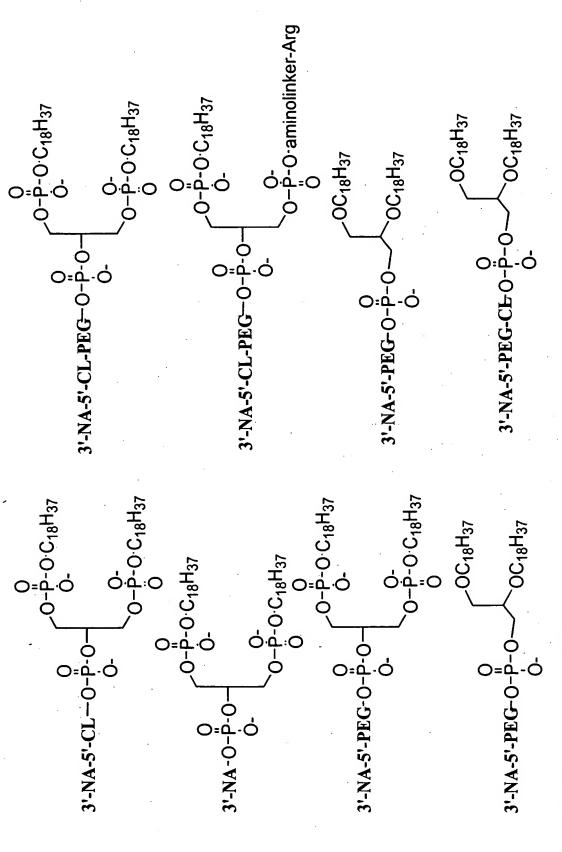
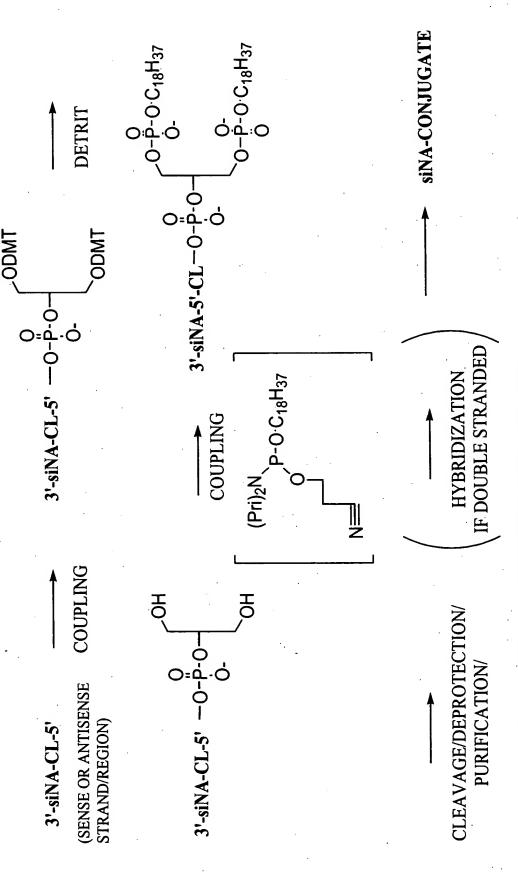


Figure 18: Nucleic Acid/Phospholipid Conjugates



NA= Nucleic Acid Molecule such as siNA, antisense, or enzymatic nucleic acid CL=cleavable linker (e.g. A-dT, C-dT) PEG=polyethylene glycol

Figure 19: siNA Phospholipid Conjugate

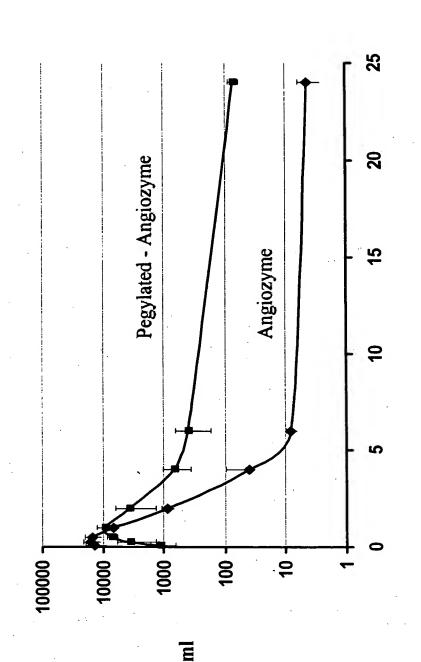


CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMIDINE DIMER THAT IS OPTIONALLY PRESENT

Figure 20: Peptide PEG Conjugate

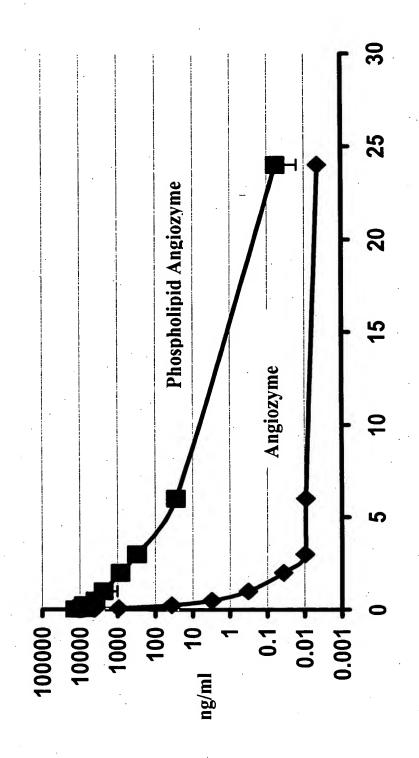
A = Adenosine

Figure 21: 40-KDa PEG-Angiozyme vs Angiozyme



Time hours

Figure 22: Phospholipid-Angiozyme vs Angiozyme



Time hours

Figure 23: Oligonucleotide-NAcGalactosamine post-synthetic coupling

FOR EXAMPLE: OLIGO-LINKER =

Where n is an integer from 1 to 20

Figure 24a: Protein with cleavable linker

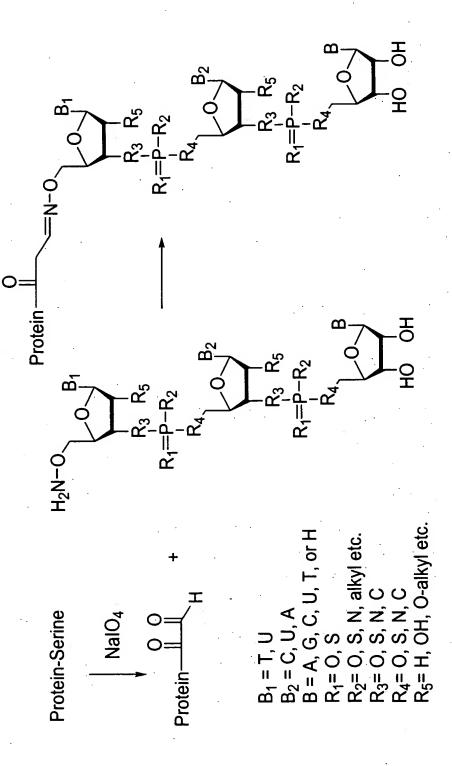


Figure 24b: Protein cleavable linker PEG Conjugate

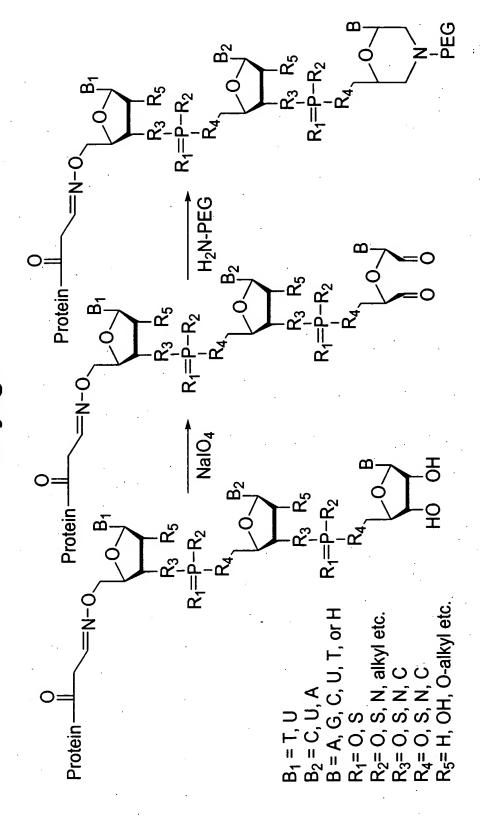


Figure 25: Protein PEG conjugate with cleavable

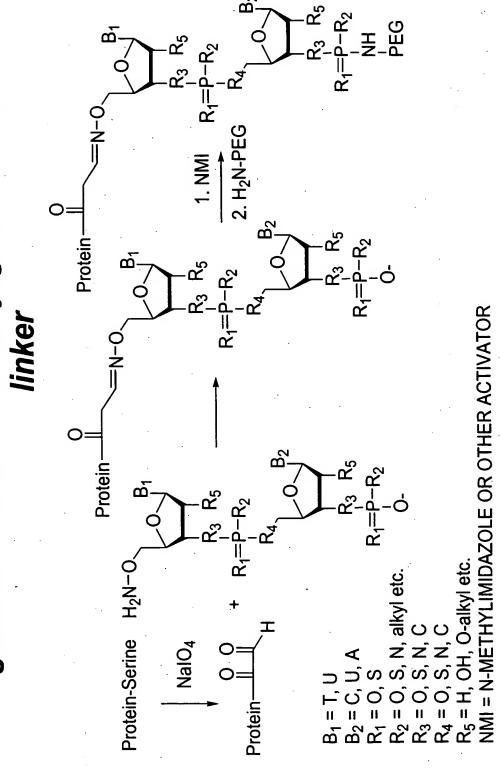


Figure 26a: PEG with cleavable linker

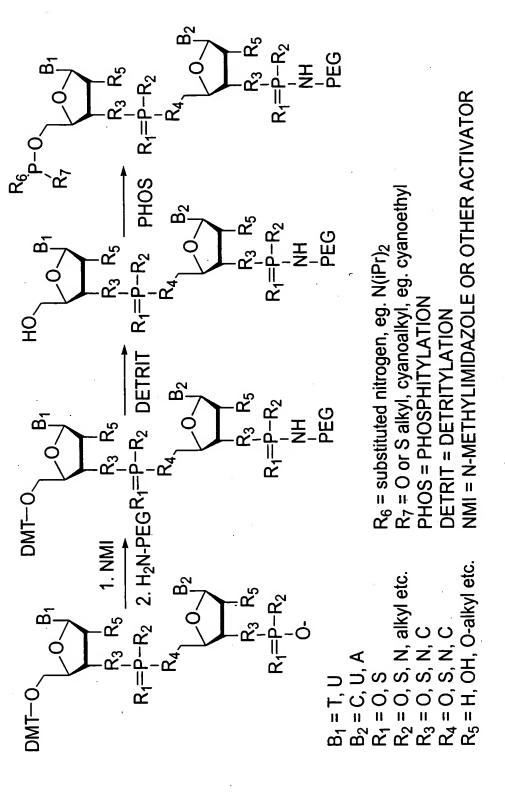


Figure 26b: Protein PEG conjugate with cleavable linker

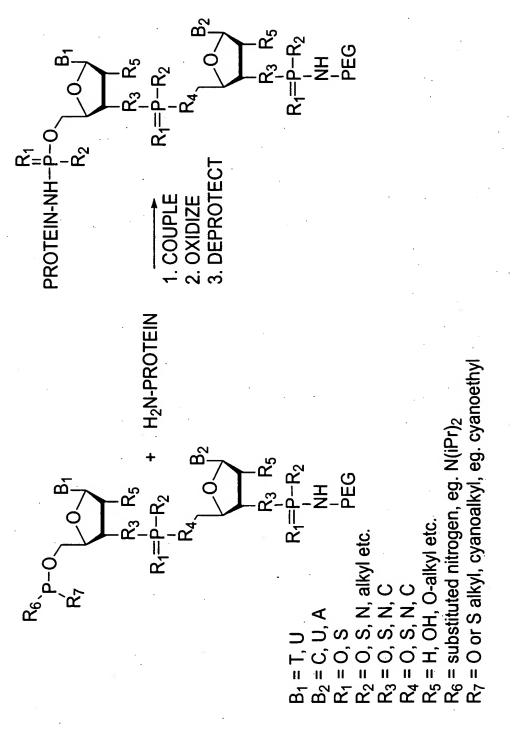
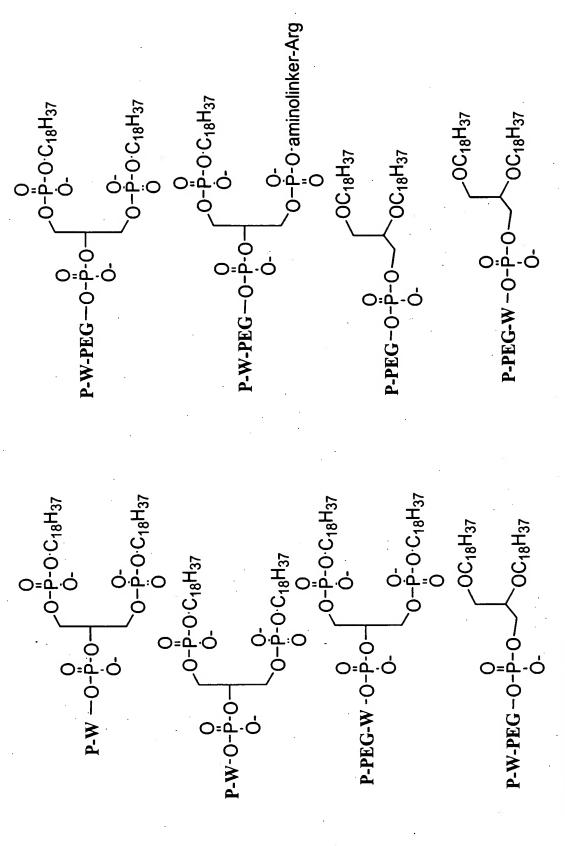


Figure 27: Peptide or Protein/Phospholipid Conjugates



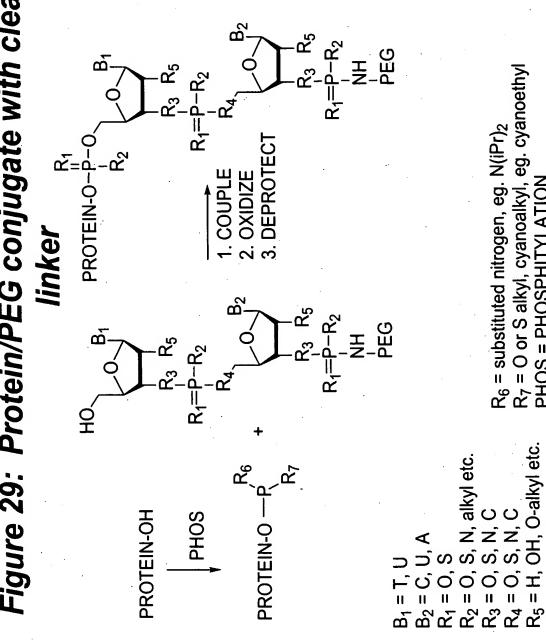
PEG=polyethylene glycol W=cleavable linker (e.g. A-dT, C-dT) P= Peptide/Protein

Figure 28: Conjugation of targeting ligands to a peptide or protein

N-acetyl-D-galactosamine conjugate

W = cleavable linker (eg. A-dT, C-dT dimer)

Figure 29: Protein/PEG conjugate with cleavable



 R_6 = substituted nitrogen, eg. N(iPr)₂ R_7 = O or S alkyl, cyanoalkyl, eg. cyanoethyl PHOS = PHOSPHITYLATION

Figure 30: siNA Cholesterol Conjugate

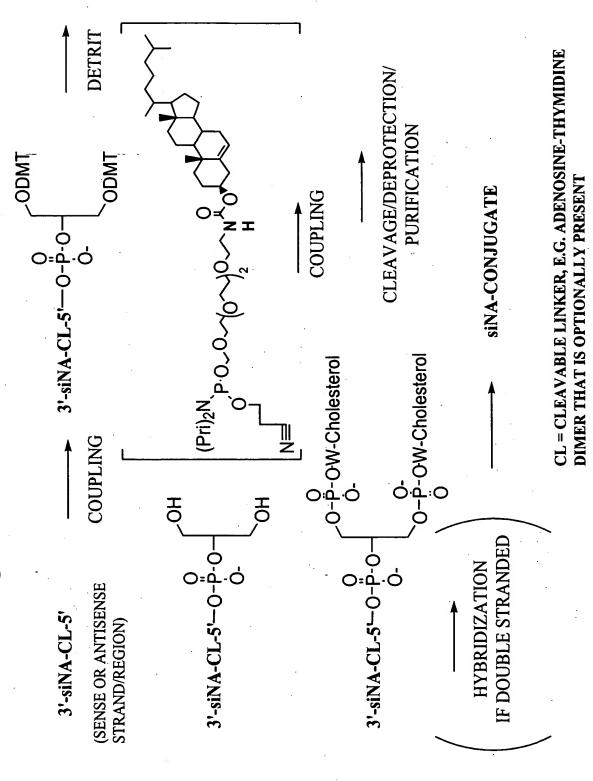


Figure 31: siNA 3'-PEG Conjugate

CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMIDINE DIMER THAT IS OPTIONALLY PRESENT

Figure 32: siNA 3'-Cholesterol Conjugate

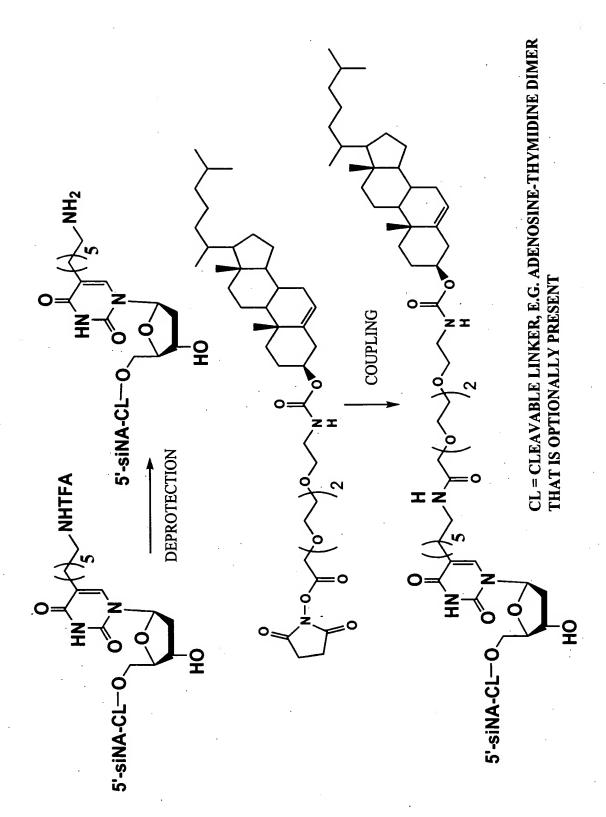
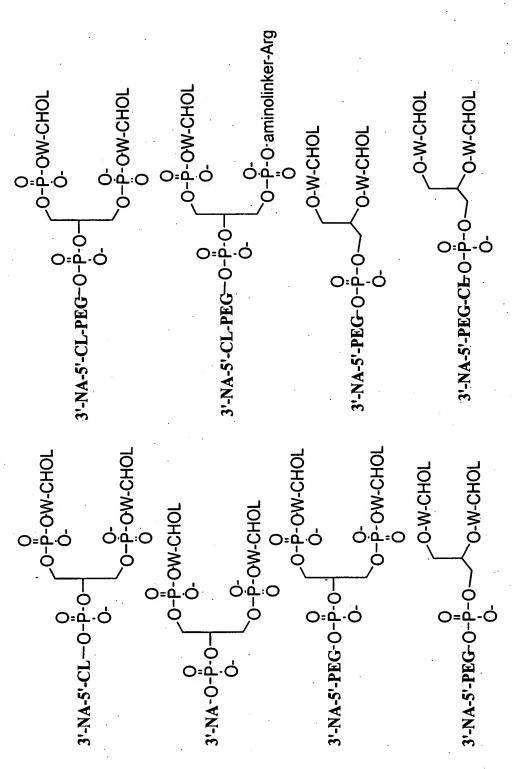


Figure 33: Nucleic Acid Cholesterol Conjugates



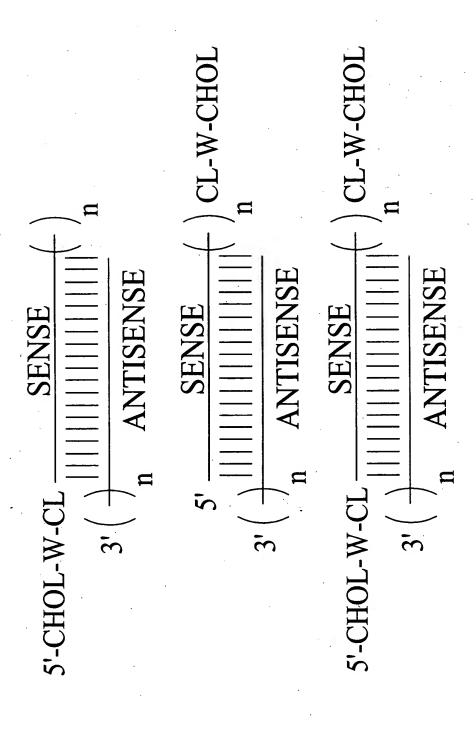
PEG=polyethylene glycol CL=cleavable linker (e.g. A-dT, C-dT)

NA= Nucleic Acid Molecule such as siNA, antisense, or enzymatic nucleic acid

CHOL=cholesterol or an analog or metabolite thereof

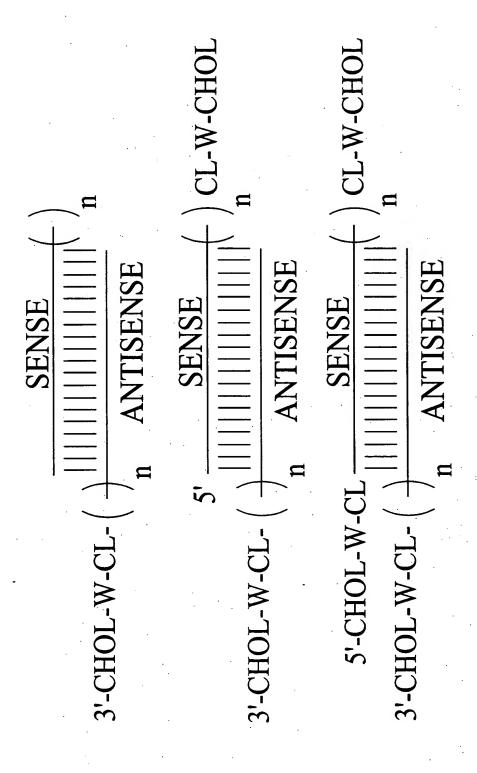
W= linker molecule (see for example Formulae 109 or 112)

Figure 34: siNA Cholesterol Conjugates



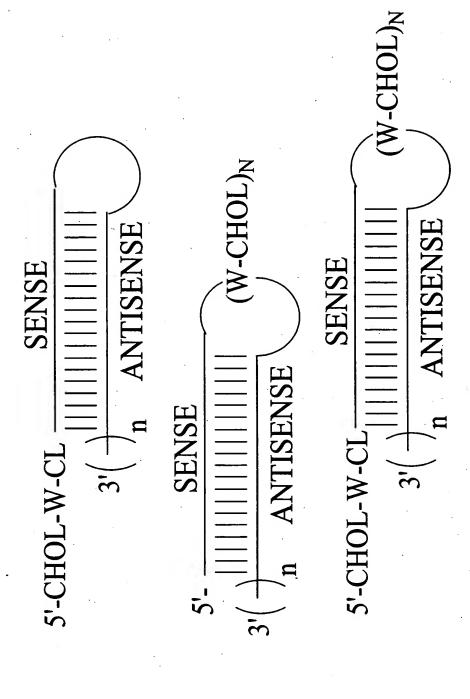
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present CHOL=cholesterol or an analog or metabolite thereof W= linker molecule (see for example Formulae 107, 108, 109 or 115) n = integer, e.g. 1, 2, or 3

Figure 35: siNA Cholesterol Conjugates



CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present CHOL=cholesterol or an analog or metabolite thereof W= linker molecule (see for example Formulae 107, 108, 109 or 115) n = integer, e.g. 1, 2, or 3

Figure 36: siNA Cholesterol Conjugates



CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present CHOL=cholesterol or an analog or metabolite thereof W= linker molecule (see for example Formulae 107, 108, 109 or 112) n = integer, e.g. 1, 2, or 3
N=integer, e.g. 1, 2, 3, or 4

Figure 37: siNA Lipid Conjugates

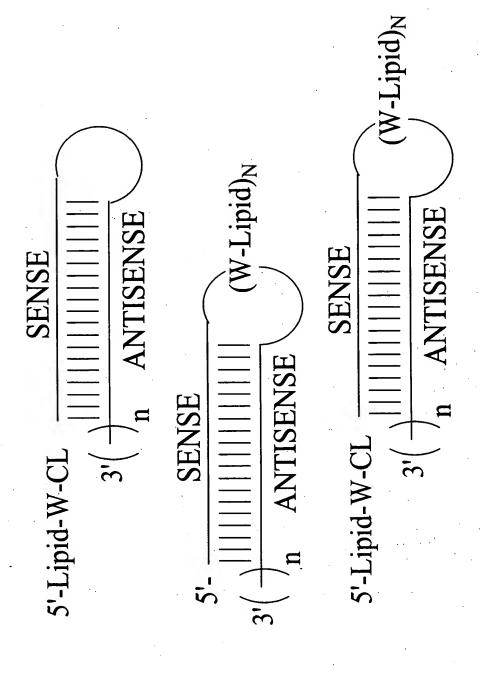
5'-Lipid-W-CL
$$\frac{\text{SENSE}}{n}$$
 $\frac{\text{SENSE}}{n}$ $\frac{\text{SENSE}}{n}$ $\frac{\text{SENSE}}{n}$ $\frac{\text{SENSE}}{n}$ $\frac{\text{SENSE}}{n}$ $\frac{\text{CL-W-Lipid}}{n}$ $\frac{\text{S'}\left(\frac{1}{n}\right)_{n}}{n}$ ANTISENSE $\frac{\text{SENSE}}{n}$ $\frac{\text{SENSE}}{n}$ $\frac{\text{CL-W-Lipid}}{n}$ $\frac{\text{S'}\left(\frac{1}{n}\right)_{n}}{n}$ ANTISENSE $\frac{\text{SENSE}}{n}$ $\frac{\text{$

3'-Lipid-W-CL-
$$\left(\begin{array}{c} 5' & \underline{\text{SENSE}} \\ \hline \\ n & \underline{\text{ANTISENSE}} \\ \end{array}\right)$$
 CL-W-Lipid

5'-Lipid-W-CL
$$\frac{\text{SENSE}}{|||||||||||||||}$$
 CL-W-Lipid 3'-Lipid-W-CL- $\left(\frac{1}{n}\right)$ ANTISENSE

CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present Lipid=Straight chain or branched alkyl or fatty acid, e.g. $C_{18}H_{37}$ W= linker molecule (see for example Formulae 48, 49, 64, or 65) n = integer, e.g. 1, 2, or 3

Figure 38: siNA Lipid Conjugates



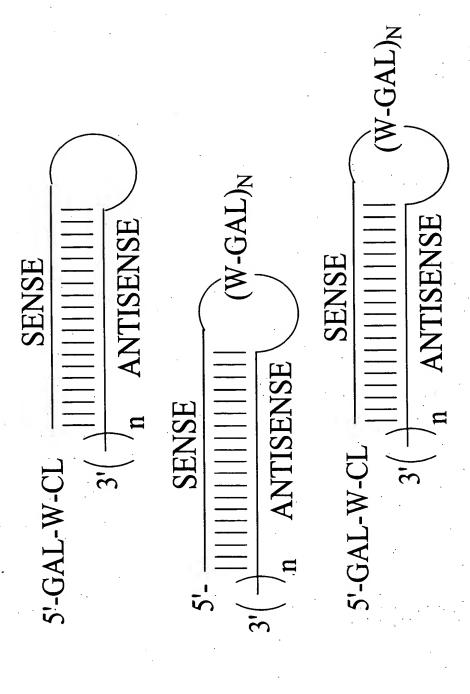
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present Lipid=Straight chain or branched alkyl or fatty acid, e.g. $C_{18}H_{37}$ W= linker molecule (see for example Formulae 48, 49, 64, or 65) n = integer, e.g. 1, 2, or 3

N=integer, e.g. 1, 2, 3, or 4

Figure 39: siNA Galactosamine Conjugates

CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present GAL=GALACTOSAMINE; e.g. compounds having Formulae 51-56, 86, 92, 99, 100, 103, 105, 106 W= linker molecule (see for example Formulae 102 or 103) n = integer, e.g. 1, 2, or 3

Figure 40: siNA Galactosamine Conjugates



GAL=GALACTOSAMINE; e.g. compounds having Formulae 51-56, 86, 92, 99, 100, 103, 105, 106 CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present W= linker molecule (see for example Formulae 102 or 103)

n = integer, e.g. 1, 2, or 3 N=integer, e.g. 1, 2, 3, or 4

Figure 41: Generalized siNA Conjugate Design

5'-CONJ-W-CL
$$\frac{\text{SENSE}}{3'\left(\frac{1}{n}\right)_{n}}$$
 ANTISENSE

5' $\frac{\text{SENSE}}{3'\left(\frac{1}{n}\right)_{n}}$ CL-W-CONJ

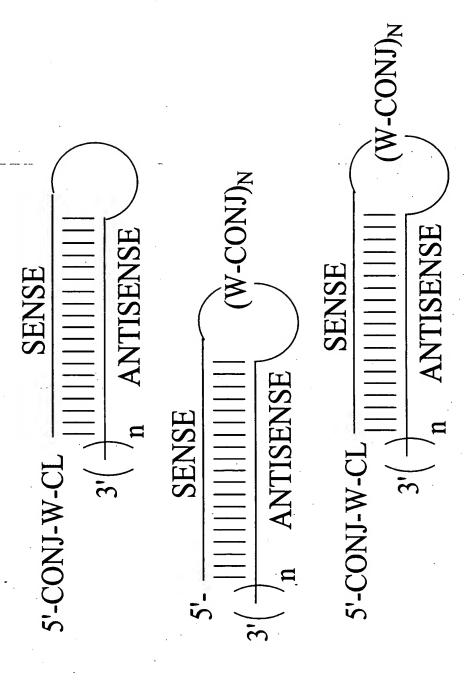
3' $\left(\frac{1}{n}\right)_{n}$ ANTISENSE

5'-CONJ-W-CL $\frac{\text{SENSE}}{3'\left(\frac{1}{n}\right)_{n}}$ CL-W-CONJ

3' $\left(\frac{1}{n}\right)_{n}$ ANTISENSE

CONJ=any biologically active molecule or conjugate as described herein CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present W= linker molecule n = integer, e.g. 1, 2, or 3

Figure 42: Generalized siNA Conjugate design



CONJ=any biologically active molecule or conjugate as described herein CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present W= linker molecule

n = integer, e.g. 1, 2, or 3

N=integer, e.g. 1, 2, 3, or 4

Administration of Conjugated or Unconjugated Chemistries Figure 43: Distribution of Intact siNA in Liver After SC

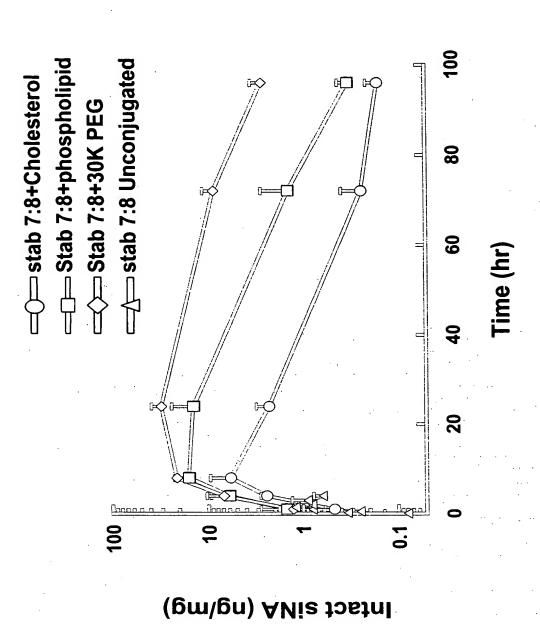


Figure 44: Lipid Free Delivery of HBV siNA Conjugates in Cell Culture

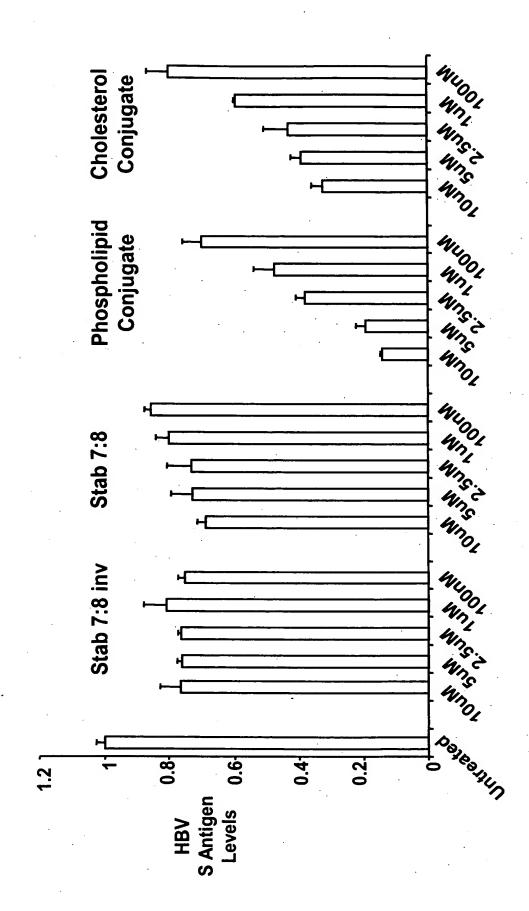


Figure 45: Scale-up of "mono" Galactosamine phosphoramidite

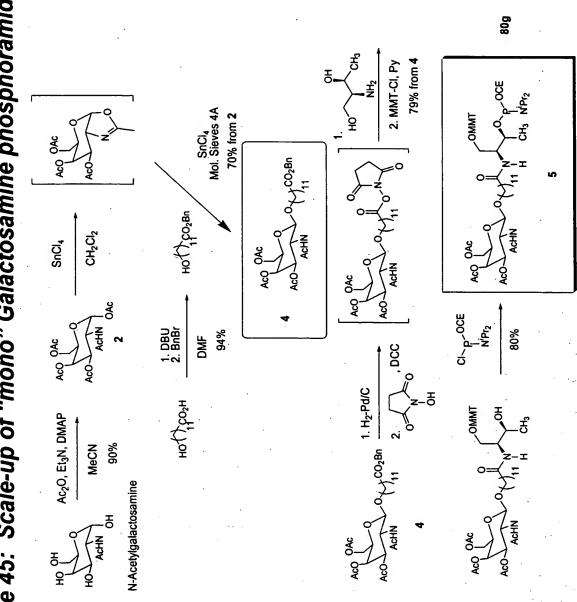


Figure 46: Synthesis of "tri" Galactosamine phosphoramidite

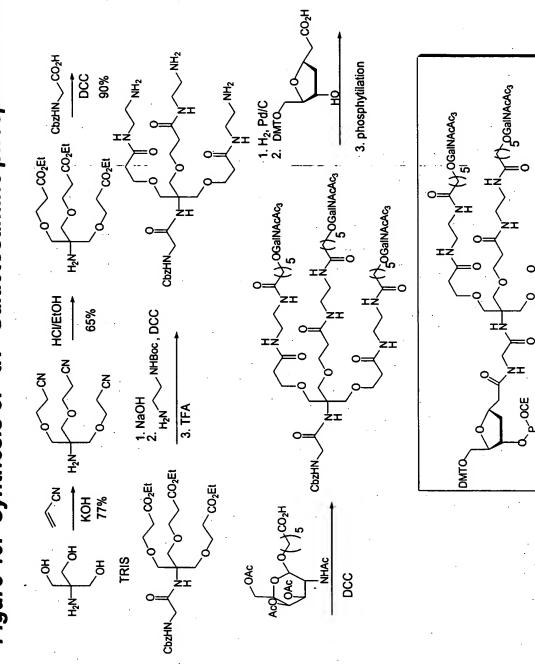


Figure 47: Synthesis of another Tri-Galactosamine Conjugate

Figure 48: Alternate Synthesis of Tri-Galactosamine Conjugate

Figure 49: Synthesis of NHS Cholesterol Conjugate

$$\frac{1) H_2, Pd/C}{2) HCI, H_2O}$$
 H_2N O CO_2H

Couple to Nucleic Acid with amino linker